Supplementary Recombinants.

Dec. 9, 1947. See 51a

Y40 x W-1.

Plate v. dilute on EMB lactose (B_1). and on EMS Mal ± B_1.

Look for sectoring. Count only clearly scored in uncrowded portions. Yields much higher on M'. from 20-100/plate.

	+	−	sectors.	
M(0).				
1.	2	32	0	
	1	38	0	
	4	32	0	
	3	25	1	v. clear sector. (1)
	12	117	1	(2)
	22	244	2	288.
2. M(B_1).	15	285	5	

seems to be fairly frequent among Mal +. However, plate is too crowded for accurate estimation.

3. Lac B_1. Yields lower than on Mal. (Mal contaminated with amac., etc?)

	+	−	sec.	
1	1	9	0	
2	17	32	0	
3	16	16	0 2?	Not clearly duplex. Maybe all Lac+.
	17	27	1?	Almost certainly not contam.
	6	33	0	
	6	18	1	v. clear
	12	21	0	
	9	14	1	v. clear (+)
	9	16	0	
	0	3	0	
	99	189	5.	

Streaks out mosaics. Test Remainder of population to get complete score on Lac, Mal + V,

Supplementary Recombinants — Maltose Segregation.

Dec. 9-14, 1947.

Y4/0 × W-1. Lac, Mal T1^R segregation.

Plate very dilute on EMS agars. & looks for sectoring (A) Scored by inspection of plates.

		Mal+	Mal−	Sectored	Sum
1. M(0).		22	244	2	268.
2. M(B,)		15	285	5	305.
Sum.		37	529	7	573
Mean.		.067	.92.50	.012	100.00.

About 8% of colonies carry Maltose +.

		Lac+	Lac−	Sec.	Σ
3. Lac(B,).		99	189	5	293
	$\bar{M}$	.338	.646	.017	

(B) Sample colonies from Lac to Mal & Mal to Lac.

B.

		Lac+ R	Lac+ S	Lac− R	Lac− S.	Σ
From 1. to Lac.						
1	Mal+.	4.		12		16
2	Mal−	45	1	66	7	119 ←

Phage scores probably unreliable from appearance of secretors. Not too good a fit with 3:1. 39% Lac+

From 2. to Lac.						
3	Mal+	10		6		16
4	Mal−	No tests.				
		Mal+ R	Mal+ S	Mal−R	Mal−S.	
From 3. to Mal.						
5	Lac+.	1	0	66	3	70
6	Lac−	0	2	89	21	112.

Compare R/s with B2!

Scores on 51 organisms.

Maltose, agar, minimal. Phage scores uncertain.

Maltose +. 12 Lac − V_1^R
4 Lac + V_1^R.

Maltose +. B, agar. 10 Lac − V_1^R.
6 Lac + V_1^R.

Maltose +. 22 Lac −
10 Lac +

Maltose −

Lactose B, agar.

	Malt+ R	Malt+ S.	Mal− R	Mal− S.	
Lac+.	1		14	1	
			7	1	
			9	0	
			~~10~~	~~0~~	
			2	0	
			8	0	
			9	1	
			7	0	
	1	0	66.	3	70
Lac− →	0	2	89	21	112.
			7	1	
			8	1	
			6	0	
			6	3	
		1	4	1	
		1	7	0	
			2	0	3 0
			15	3	15 4
			8	8	6 5

M –

Lac+ R	Lac+ S	Lac– R	Lac– S
12	5	5	
		2	
3		1	2
3		3	
5		9	1
6		9	
3		7	
4		4	1
4	1	7	1
2		10	
3		8	1
		1	1
45.	1	66	7

Phage sources unstable

Cumulative data on maltose:

a +:-	−	+
	272	15
	244	24
	285	20.
	801	59 / 860.

M+ = 6.8%.

Lac+:- in Mal +: ~~15~~ : 13 Lac+ : 9 Lac −
22 + : 10 −

35 Lac+ 19 Lac− / 54

The 2.4% triples compared to 4.4% singles imply a map distance ca. twice that found on the basis of the Lac, V data. A crossover in the Mal Region may, by interference (a) ~~coincide~~ favor additional crossovers to make unrecoverable that chromatid, or (b) augment the relative frequency of triples.

Dec. 9, 1947.

Spread W-1 and T1 on EMB-Maltose plates to select for spontaneous T1 resistant mutants.

Numerous, well-defined smooth W-1/1 found.
Streakout one such colony to provide (W-54) derivative. (1)
Test 70 others. all ~~#~~ resistant to T5.

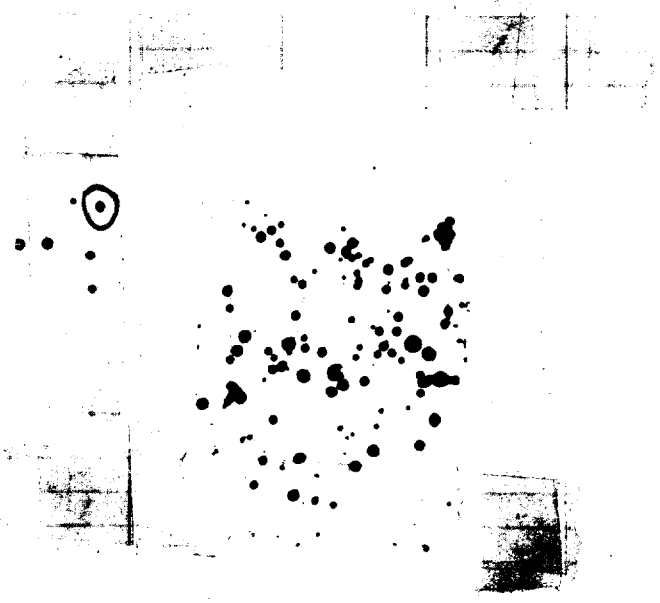
Dec. 9, 1947

W-45 (Lac_2^- B_1^+ Lac_1^+ × Lac_2^+ B_1^- Lac^- $Mall^-$)
×
W-1.

Plate dilute on B, EMS Lac.
Numerous Lac+.

+	−	
32	68.	/100

plates stiff scatter crowded in some cases.
Some probably valid sectoring noted.



Streak out Lac− on Lac agar to look for reversible Lac− types.

Pick single colonies to maltose plates to score Mal and to provide cells for reversibility test.

of 42 Lac− tested, #41 Mal−
1 Mal+

Test all of these for Lac reversibility on lactose .05% medium.
All marked reversible turbidities with heavy inoculum. Test by loopful streak on Lac EMB. all+ except number 5.
Keep as (53-5) If this is irreversible, regard as Lac_1^- Lac_2^- and test by recombination tests.

W-1 53-4 results!

Streak out, on EMB Lac all produced papillated colonies, although only one rec. colony of W-45 was papillose.

53-4
53-5
53-6 – note that two colonies were non-papillogenic. Retest!
Y53
W45

Dec. 9, 1947.

Irradiate .1 ml Y40 per EMB plate, 20 plates 10 secs. u.v. Hanovia lamp

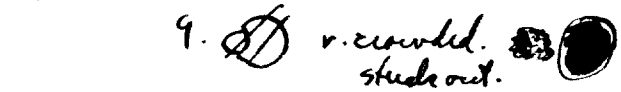
Irradiation by D.G.

Lac. 59 plates. ca 200 readable colonies/plate = 12000.

1. Crowded plate  maybe juxtaposition. 8. 

2.  v. clear sectoring.

3.  debris.

9.  v. crowded. streaks out.

4.  small colony.

5.  v. small crowded plate

6.  both pure white. separated.

7.  v. small.

Mal. 45 plates. = 9000

11.  Tiny sector, scarcely distinguishable.

12.  [redacted]sector "ws" at best. Streak out entire colony

13.  clear, small sector. Streak out vicinity of sector.

14. sector indistinct

15. 

16.

17.  sector indistinct.

18. Intact white.

19. Intact white

20. Intact white

21. 

22. Like 12.

23. 

24. O intact white

25. 

Classification of Mal + Lac mutants.

Dec. 13, 1947.

11. Dark + dull blue colonies noted. Streak separately in effort to find destinction

12. Some colonies light brown in transmitted light. As above.

13. Mostly – W56 Some + W-57. Also some v. light ± Restreak these.

14. Some definitely ± compared to stand. Re.

54-15 Remic.

54-16 Remi.

17 No marked variation.

18-20 Remi. 18: W60
19: W61
20 W62

22. As 11.

23. as 11.

25. Mostly –. A few + in sectors. – W58
+ W59. Restreak.
+'s in form of sectors.

Dec. 15, 1947.

54-11 ~~Restreak~~. Reject.

54-12. No difference noted.

54-13. 3 types noted. – W56 } Repeat comparison.
+ W57 }
± W63 }

54-14. a) Mal ++

b) Faded in part of streak. Reject.

15. a Turned Blue. Restreak c = Mal- b like a. restreak. W71.

16. Restreak.

21 Restreak

22. Restreak.

23 Restreak.

24. W64.

25 W.59 (Mal+"). Vc sectors. Pick b slant.

---

1. + and - colonies. – W65
+ W66

2. 2+ 2- sets. – W67
somewhat mutually – W68
contaminated. + W69
+ W70

3. Indistinguishable pairs

4. ~~Test~~? + and – – W72
+ W73.

5. ~~Test~~? + and – – W74 + W75.

~~6. a + b. W76 W77. May be sibs.~~

7. + and – Mostly –. – W76
+ W77

8. Restreak.

9. all +.

Dec. 16, 1947.

-16 two types of colony.
a. + W79
b. - W78

22- a. + W81
b. - or ± W80 + weak? Definitely less than c.

8 - a Mostly + colonies. W84
b. = Apparently - colonies } Pick all of these W83
c. " " ". W82

23. Indistinguishable.
c. Indistinguishable

15. b. = Mal+. W85.
a. + and -. Probably mixture. - W71a.
c. = W71

Lac − alleles by Lac reversion.

Dec. 8, 1947. and earlier.

Streak out various Lac − strains on synthetic Lac agar to select for reversions. Streak from reversion colonies directly onto β-Φ galactoside and read after 48 hours. Original responses from previous data.

		or.	a (Reversions.)	b	c	d	e	f	g	h	i
1.	Y53.	+	+	+	+	+					
2.	W35	−	±	±	+	±	±	+	+	±	±
3.	W30	+	+	+	+	+	+	+	+	+	
4.	W36	−	±	±	±						
5.	W40	+	±	±							
6.	Y87.	+	+								

Isolate on Lac EMB the W35 series.

2: a. Only an apparently "weak" positive on lactose. Keep.
b. Lac ++.
c. Mostly ++. Some Lac −.
d. Lac ++
e. Lac −
f. Lac ++.
g. Lac ++
h. Lac −
i. Lac −.

Types.

		Bφ	Lac
a	:	±	−
b		−	+
c		+	+

Compare:

		Bφ	Lac
58-161	~~[illegible]~~	+	++
W-35	~~[illegible]~~	±	−
a		±	±
b		±	++
c		+	++
d		like b	
f.		like c	
g.		like c	
~~h, i.~~			

Keep on slants.

Also compare ē W-35 on Lac ~~[illegible] EMB.~~

Dec. 15, 1947.

Y132. in Arginine T(0) +: Inoc. P15.

		A16
1.	—	—
2.	—	—
3.	NEA	—
4.	EAA	—
5.	N+E AA	—
6.	HC	—
7.	Y Ex	+++
8.	Vits	—
9.	HC+Vits	—
10.	Glycine	—

Yeast Extract Mutant?? try N2 case, Nucleic acid.

45-1, 2, 3 in: Valine T(0) +

	1	2	3
1. —	±		—
2. EA (circled)	+++	not coli	—
3. NA	+		—
4. Vits	±		—
5. Pur+Py.	±		—

maybe reverting. see 46.

No growth in any of these! W-1

1st reading A16

is mutant. Grows on T(0), + all T(+ ess. amino ac.) except isoleucine which seems to inhibit it! Inc. at 22-28°. (surface of water bath at 37.

U-1A. on T(0) +

	A16	A18
1. Y. Ex.	—	++
2. Vits.		++.

W-1 on T(0) +

		A16
1.	—	++
2.	~~2.5~~, .25 μg/ml CAB (2-chloropab from Strandskov)	+ ~~+++~~
3.	do. + methionine 10γ/ml.	+++
4.	do. + pab .1γ/ml	+

YNA. Preparation. Dissolve 25 g. Schwarz Nucleic acid in 125 ml H_2O + 11 ml 28° NH_3 water in 500 ml flask. Wire Stopper and autoclave at 15 lbs.

Maltose mutants on trehalose

Dec. 15, 1947.

Test the following on trehalose E17B. 16 4.

		Tre.
Maltose + :	Y40.	+++
Maltose − :	W1	+++
	W3	+++
	W21	+ weaker.
	W60	+++
	W61	+++
	W62	+++
	W56	+++
	W64	+++
	W58	+++.

Trehalose is, therefore, attacked by Maltose negative mutants. Cross adaptation should be checked. ✓

		Tre	Mal
Dec. 18, 1947.	W-63	+	+
	W-71	+	−
	W-78	+	−
	W-80.	+.	+

all maltose-negative mutants so far found are Tre- +.

Nutrition of Y132.

Dec. 17, 1947.

T(0) +.

		A17 (16h.)	A18.
1. Y. Ex.	.5%	+++	✓
2. "	.05%	++	✓
3.	.005%	±	✓
4.	.0005%	−	✓
5. Y.N.A.	.5%	−	++
6. N2Case	.5%	±	+
7. —		−	−

N2Case is much less active than yeast extract.

YNA has some activity - only ca. .1 − .01 of yeast extract.

Try Casein, Acetate, other protein hydrolysates, e.g. gelatin; lactalbumin; fat-soluble incl. oleic acid.

T(0).

1. −
2. Y. Ex. .1%
3. N2-Tone .5%
4. N2Case .5%
5. N2AmineB .5%
6. N2AmineA .5%
7. Casein .5%

Suspend 10g. Y. Extr. in ca 30ml $CHCl_3$. After 1 hr. filter. Evaporate $CHCl_3$ from extract and take up in # 20 ml H_2O. Do. Residue, taking up in 200 ml H_2O.

Lac-1 and Lac-2 mixtures

Dec 18, 1947.

Make up 10 mm tubes of lactose 1% BCP broth.

	Add .5 ml inocula of :	6 P 16	Set up 2P16 10 A 17	48	A18	
1	W-45	-			++	*
2	W-45	-			++	
3	W-54	-			-	
4	W-54	-	The Same		-	
5	W45 & W54	-			++	*
6	W45 & W54	-			++.	
7	K-12	///				
8	K-12	///				

Therefore mixtures of Lac-1 and Lac-2 are unable to utilize lactose, although recombinants are able.

* Streaked on lactose. Probably revertants
Mostly + colonies. Streaked out to get W-45^R for allelic tests.

Dec. 17, 1947.

Harvest ~~x~~ W-45 (Mal+ Lac-1+ Lac-2-) ~~x~~ and W-54(Mal-Lac-1-Lac2+ V_1^r) from fresh YP cultures, and mix at a conc. ca 10^{10} /ml each in water. Store over night in refrigerator. Dilute to 10^3 / ml. and spread .1 ml on EMB-Lac (NZCase) plates to ~~detect~~ possible Lac1+Lac2+ recombinants.

12/18 PM. 111 plates x 357/2 totalling ca. 40,000 colonies examined. None were Lac+. This is a control on the reversion of both Lac-1 and Lac-2 The recombination rate under these conditions is apparently too low.

Nutrition of Y132

Dec. 18, 1947.

Inoc. into T(A) ∠

	A19.	P19.
1. Y. Extr. .5%	+++	✓
2. Y. Extr. .05%	++	✓
3. YX Residue .5%	+++	✓
4. YX Residue .05%	++	✓
5. YX Extract .5%	±	bacteria in initial extract.
6. YX Extract .05%	–	✓
7. Gelatin Hydrolysate .5%	±	+
8. Tomato Juice .5%	++	✓
~~9. Casein .5%~~		
10. NZTone .5%	+++	✓
11. NZAmine A .5%	+	++
12. NZAmine B .5%	±	±
13. YNA intact (NaNucl.) .5%	±	±
14. YNA hydr. .5%	–	–
15. YNA hydr. .5% + YX .5% test for inhibition.	+++	+++
~~16. xxxxxxxxxxxx~~		
16. --	–	–
17. NZ case .5%	±	+++ (adaptation? or auxin?)
18. Oleic acid .001%. Free acid still at surface.	± - +	turbidity not due to bacteria.

[Try Tween, Na oleate, etc. (Acid extract of fresh yeast!)] [Maybe in NZ case?]

10g. Yeast Extract Difco extracted with 40 ml. $CHCl_3$ in flask. Separate, evaporate extract and take up in water. Expressed in terms of original yeast content. (Very little material was extracted, perhaps 1-5mg. at most.)

W5-3 in T(V) +

		P19
1. –		
2. YX .2%	++	++
3. HC+V	+	++
4. NZ case	–	++.
5 YNA		

2-Chloro-4-aminobenzoic acid
Inhibtion and resistance mutations

Dec. 15, 1947.

Prepare plates of T(0) agar with 25 mg% CAB. Do. (0) agar.

Spread ca 10^2 cells of K-12 on both, incubate 72 hrs.

A) T(o) agar: 400 colonies noted

b) CAB: ca. 42 colonies noted. However, direct microscopic observation and smear impressions show a large number of "micro-colonies", probably equivalent to the difference between CAB and T(0) plates. Each colony contains, as a guess 10^4 - 10^5 cells.

(This suggests that Strandskov's observations can be accounte d for on the basis of spontaneous mutation and selection among the relatively large numbers of cells in the micro-colonies.)

Dec. 16, 1947.

Harvest from YP and cross W-55 x W-54, heterozygous for Lac_1, Mal, Sal,B_1 as well as V_1^r. Cross on EMS-maltose with .002% glucose added. $+B_1$.

A20. a) Estimate frequency of maltose+, and of sectored colonies. Score only those where the sectors could be scored accurately.

Proportion of Mal + (including sectors). Count sectors as 1 + and 1 −.

	+	Sec.	−
1	1	2	57
2	4	0	139
3	3	1	117
4	2	0	77
5.	7	2	132.
	17	5	512

$\frac{Mal+}{Total} = \frac{17+5}{512+5+17} = \frac{22}{534} =$

4.2%.

Proportion of sectored to plus colonies: (Score under conditions stated above)

Plate	Sec.	+	#	Sec	+	#	Sec	+
# 14	2	6		1	5			
	0	3		2	3			
	0	2		1	4			
	1	6		0	3			
	1	1		1	1			
	0	2		2	1			
	0	4		0	2			
	1	1		1	4			
	0	2		3	3			
	0	1		1	7			
	1	1		4	1			
	0	3		1	2			
	2	4		0	4			
	2	4		1	3			
	1	3		0	2			
	4	2		2	2			
	1	4		2	3			
	1	3		2	3			
	1	1						
	0	4						
	2	2						
	1	3						
	0	2						
	0	1						
	2	3						
	1	1						
	1	2						
	1	1						
	2	2						
	0	2						
	0	1						

52. 130 / 172.

30% of the Mal+ colonies also have a Mal- segregant.

a20

Score Mal- segregants re Lac and V_1. Also Score Mal+

Mal-	Lac+ V^R	Lac+ V^S	Lac- V^R	Lac- V^S
	0, 1,	10, 9,	5, 4	4, 2
Mal +	Lac+ V^R	Lac+ V^S	Lac- V^R	Lac- V^S

(Not scored well on Lac) too heavily contaminated with parental to score on EMB. Recover Mal+ from these plates & test on EMS.

Obtain new sample of Mal- from cross plates.

Pick 57 apparently sectored colonies to water N20. Store in refrigerator for later separation. Streak out on Mal.

Scores on Mal p/m components of maltose sectored colonies.
Lac p/m and V_1 r/s

Colony	Mal p	Mal m
1	ms	ms
2	ps	ps
3	ms	ps
4	ms	ms
5	ps	ps
6	ms	ps
7	ps	ps
8	ms	ms
9	ms	ms
10	mr	ps
11	ps	ps
12	ps	ps
13	mr	mr
14	ms	ms
15	ms	ms
16	ps	ps
17p	ps	ps
18	ms	ps
21	ps	ps
22	ps	?s (m)
23	ms	ms
24	ps	ps
25	ms	ms
26	ms	ms
27	ms	ms
28	ms	ms
31	ms	ms
32	ps	ms
33	ms	ms
34	ps	ps
35ms	ms	ms
36	ps	ps
37	ps	ps
38	ms	mr
39	ps	ps
40	pr?	ps
41	ms	ps
42	ps	ps
43	ps	ms
44	ms	ms
45	ms	ms
46	ps	ms
47	ps	ps
48	ms	ms
49	ps	ps

Scoring very clear except where total lysis may have obscured fermentation reading in 22

Totals: 45 tests.

	Mal +	Mal −
− S	23	~~20~~ 22
− R	~~20~~ 22	
+ S	20	
+ R	1	

	Mal +	Mal −
− S	22	21
+ S	20	22
− R	2	2
+ R	1	0

M+ ↓ / M− →	−S	+S	−R	+R.	Σ
−S	17	4	1	0	22
+S	4 ~~20~~ ~~18~~	~~4~~ 16	0	0	20
−R	0	1	1	0	2
+R	0	1	0	0	1
Σ(M−).	~~24~~	~~20~~ 22	2	0	45.

Compare − and + only.

	M− (L−)	M− (L+)	
M+ (L−)	19 [12]	5 [12]	24
M+ (L+)	4 "	17 "	21
	23	22	45.

$\chi^2 = 16.2$

$p \ll .001$.

∴ There is a definite correlation between the Mal− and Mal+ components of sectors in re Lac segregation. Recover pairs to Mal plates.

(Y54 x Y55.) . Mal-Lac,- V_1^R x Mal+Lac,+ V_1^S.

Lac, V_1 scores of intact colonies:

A) Mal +

−R	−S	+R	+S.	
5	4	0	7	
2	7	0	9	
3	4	0	10	
2	4	0	1	
12	19	0	27	/ 58.
21	32	0	47	

B) Mal −

5	7	1	10	
7	5	0	10	
5	6	2	5	
4	7	0	10	
4	3	0	11	
25	28	3	46	/ 102
35	39	2	64	

37. 47 3 73 160

[23.2] [29.4] [1.9] [45.7]

$\chi^2_2 = .85$. $p = .6$

Compare with Table 5 of Kimura's paper. Ratios were.

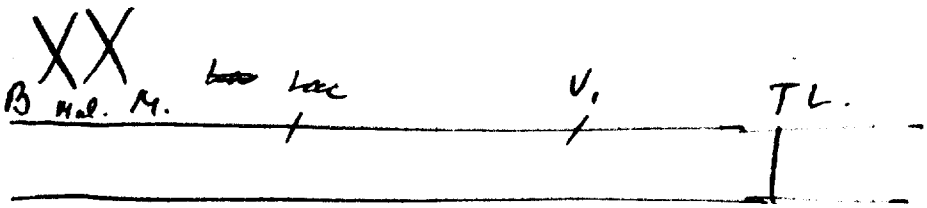
Is this medium keto-sufficient?

Mal must be between M and B. (no interaction with Lac).

Compare with sectored colonies:

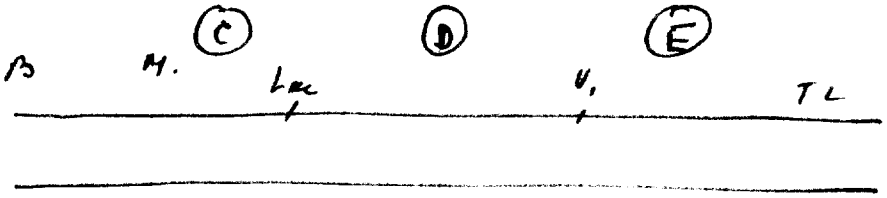
Intact.	37	47	3	73	160.
Sectored	46	43	1	42	90.

Note: Absence of -R and deficiency of +S.



a) a double crossover in Bal region does not interfere with chromosomes to the right - homogeneity of Mal+ / Mal-.

b.



If a double takes place, it tends to be both in region D or both in region C. Very few doubles are recovered involving region E.

Mutagenesis with GE 100W AH-4 lamp Unit.

Killing curve.

Ded. 24, 1947.

Spread .1 ml. young (ca. 10^9) 58-161 culture on EMB-Lac plates. Irradiate at 25 cm.

Lamp borrowed from Stauffer. operated in horizontal position, with 10 mins. warm-up allowed. This lamp has section of glass cut out to allow unfiltered uv radiation.

	Time	Surv.
	5s	~37L + 33S. = 70. ca. 10^4
*	10s	ca 100L + ca. S. 200.
*	15s	32L + ca 100S. = 130. Including white colony. Streak out + test with T1
	20s	17L + 26S = 43
	30s	13L + 9S = 22

Are these curves really non-linear? What about resistance of residuals?

Large and small colonies noted. Restreak for mix. determination;

* Reject as probable contaminant: not lysed by T1.

The difference between large and small colony noted on the above survival plates breeds true on the first restreaking on EMB-Lac. Transfer to slants as 67-1 and 67-2 for the large and small respectively.

Dec. 25, 1947.

Use same cells of 58-161 as in Exp. 67. Expose .1 ml culture per plate to 15 secs. UV from GE AH-4 lamp, as in 67. EMB - Trehalose plates

A). 9 plates.

Most plates, due to faulty pouring, had pitted surfaces and were very unsuitable for scoring. 1 plate, prepared earlier was more satisfactory. This had many (5-10%) colonies which had radial striae suggesting numerous variations affecting intensity of fermentation. Hold plate in refrigerator for later testing.

1/3/47. No mutants found.

Dec. 25, 1947.

Set up as in 68. EMB Mal plates.

Ca. 11 x 200 or 2200 colonies. 1 sector noted. Pick to slant as 69-1 for later verification.

large and small colony types seen as above.

Jan 2/48 Streaked on EMB ~~Mal~~ Mal

all Mal+. No mutants

Dec. 26, 1947. Set up as 68. Lac-EMB plates.

Killing variable. Ca. 200 scoreable survivors per plate average 65 plates, or ca. 13,000 colonies examined. 5 possible sectors.

1 Mucoid

2 Not sectored

3.  No mutants. All lac+

4.  + and −. − W87 + W88

5.  No lac mutants. Many mucoid.

Pick to slants

Jan 2, 1948. Streaks out on lact EMB.

Dec 27 1947.

Grow W-55 (58-161 Salicin pos. mutat,) in YP broth, harvest and conc. to ca. 10^{10}/ ml. Irradiate 3 ml. in quartz flask ca 5 secs. rotating at ffont of Hanovia UV lamp, and inoc. ,5 ml samples into 50 ml T(m) with .05% sugar.

1. Unirr.

   A. Salicin(.1%) 3+
   B. Cellobiose -
   C. b-Me Glucoside 3+/

2. B. Cellobiose -
   C. b-Me-Glucoside 3+

Incubate 37°

Examine Jan 3 48.

Streak out the b-MeGl cultures on similar ~~xxx~~ EMB plates. This may be slow rather than mutative utilization ,a s has been observed before.

No rapid utilization indicated on EMB - b-me glucoside plates. Growth assumed to be due to slow continuous utilization.

Raffinose tests continued from previous experiments; some possible diversity in progeny of repeated selections. Compare streaking of a - and ± colony.

A8. (3da.) + types are somewhat more purplish than - . Buds time on this streaking and all colonies are, more or less, ± coreable. Inoc. into .15% T(m) - Raffinose to continue selection. No growth.

A15. 1B (Cellobiose) noted to have reached ++± while 2B is still ±. Streak out on EMB Cellobiose agar to isolate possible mutant.

A13 Inoc. melibiose Tp (BM) ± 58-161 to select more rapid Mel+ types. Streak out and compare with standard.

Jan 4 1948

OK. W-1 X

A 8 (proportion +).

Parentals.

1	W-56	-	All −.
2	W-58	+	ca 1:10 Mal+
3	W-60	+	ca 1:20 "
4	W-63 (Z?)	+	< 1:10. Parent Mal+.
5	W-71	+	ca 1:50
6	W-78	+	ca 1:50 Mal+.
7	W-80 ±	++	
8	W-20	+	ca 1:50

W-63 × Y53 all + Parent +.

W-80 × Y53 all +

([illegible] × Y53.).

a) W-1 & W-56.

b) W58, W60, W-20, W-71, W-78.

All + recombinants checked and definitely ++.

checked as parentals.

	−	+
W1.	OK.	
W56.	OK.	
W58.	OK	
W60.	OK.	
W71	OK. Numerous papillae. Streaked out single colony.	
W20.	OK. Many mycoid. W-20 may be faintly +.	
W78.	1 Mal+ colony / 200 Mal −. May not be purified. Streaked out.	

Jan. 5, 1958

Irradiate Y-53 on Tre-EMB plates, 10 sec. under Hanovia UV lamp. 10 plates.

10 x ca 150 = 1500 colonies.

2 colonies showed fairly distinct sectoring.

1. 

2. 

Restreaked.

① and ② both give two colony types:

a.  Extensive darkening; no sheen W-89 and W-91

b.  central spot only. (This may be due to hydrolysis.

W-90 W-92

2. colonies showed radial striations suggesting quantitative variation. This is correlated with green shiny and from colony, sheen and sheen-colonies noted. The original population is very variable in this character.

Test on Maltose:

89	++
90	++
91	++
92	++.

Retest on Tre EMB. (studying Trehalose by filtration).

Jan 5, 1948.

3P. Inoc. Y105 into YP broth to obtain cells.

~~Lamp Broke Down~~

Use Stauffer lamp. Irradiate 15 secs. (Y105 is apparently more sensitive to UV than is Y10)

Mal EMB 37 plates x ca 20/plate. = 700 colonies. ~~No mutants~~

Lac EMB 38 x ca 20 = 750 colonies.

1 white colony noted. Streak out on Mal and Lac

Mal - ok. Lac + and morphologically identical with other types.

W-94.

Nutrition of W-93

Jan. 9 1947

Inoc from fresh slant into:

T(Val) plus:

		48h.
1.	-	—
2.	HC	+
3.	Vits	-
4.	HCVits	++
5.	NZVits	+++
6.	HCV+ YNA	—
7.	Y. Extr	++++

~~xxxxxxxxx~~

Looks for sp. vitamin.